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Report Title

Final Report: Solving Inverse Problems for Mechanistic Systems Biology Models with Unknown Inputs

ABSTRACT

The goal of the proposed project is to develop and test the feasibility of a novel approach to solve the inverse problem for a class of systems arising from systems biology study, in which input is unknown (e.g. cannot be observed) but multiple outputs can be observed. As mentioned above, the most significant challenge associated with this class of inverse problems is that the standard approach to inverse problem (where the parameters of the mechanistic model are optimized to fit the input-output data) is not applicable in the absence of system input observation. To resolve this challenge, this project proposes to exploit the commonality shared by the outputs that originate from the same input. Indeed, noting that these outputs originate from the identical input, a relationship between these outputs can be formulated, which can subsequently be utilized in solving the inverse problem without necessitating the input observations.

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10/03/2014 1.00 Nima Fazeli, Chang-Sei Kim, Jin-Oh Hahn. Data-Driven Modeling of Pharmacological Systems using Endpoint Information Fusion, Applied Mathematical Modelling (08 2014)

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Ramin Bighamian	0.55	
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FOREWORD

Personalized medicine is a future paradigm of healthcare that can offer right treatment to the patient at the right time to achieve safer and more effective treatment of diseases [1]. Identified as one of the Grand Challenges for Engineering in the 21st century by the National Academy of Engineering [2], it is a goal that today's biomedical engineering community strives to fulfill in order to make ground-breaking changes to current medical services offered as "one size fits all," such as titration of opioid for pain that is guided by expert opinion and experience [3] and vasopressor therapy titrated to mean arterial pressure greater than a fixed threshold value [4].

Systems biology can play a crucial role in expediting the paradigm shift from current reactive medicine to predictive and preventative medicine for disease diagnostics and therapy [5]. Considering that today's personalized medicine still relies heavily on manual drug titration (e.g., pain medicine [3] and vasopressor [4]) and population-based therapeutic index/drug levels (e.g., cancer [6], [7]), and also as evidenced by recent initiatives arising in academia (e.g., [8]), technological leap in systems biology including pharmacogenomics, pharmacokinetics, pharmacodynamics etc. can catalyze fundamental changes in the next-generation healthcare, by predicting the risk of disease development in an individual and providing personalized treatment recommendations such as automated drug selection and dose adjustments.

The fact that mathematical models are a vital component of systems biology highlights the significance of the inverse problem. The inverse problem is a gateway to unveil the nature of a biological system from the understanding (e.g., through observations) of the overall behavior of the system. As such, it plays a crucial role in (i) testing the relevance and validity of a mathematical model proposed for a biological system/process by fitting the model to observations, and also in (ii) actually benefiting the personalized treatments of combat casualties and civilians with wounds and/or diseases by individualizing the validated mathematical model (i.e., adapting the model to each individual). Therefore, the ability to solve the inverse problems for mathematical models used in the systems biology study (e.g., mechanistic models) can potentially enhance our understanding of complex biological processes (by testing the relevance of a range of empirical and mechanistic models) and also contribute to the real-world healthcare through personalizing medicine and therapy.

Traditionally, an inverse problem has been formulated so that an assumed mathematical model is fitted to an input-output data pair observed from the target biological system/process. However, there are situations where the availability of input-output observations is limited. In particular, there is a class of biological processes in which only the outputs can be observed but not the input(s). For instance, in cancer chemotherapy, the effect of anti-cancer drug on tumor growth can be understood by identifying an input-output relationship (or model) between the drug concentration and the resulting tumor size. However, taking drug concentration measurements directly in the tumor cells during the therapy is extremely difficult [9]. Other drug therapies, including pain medicine, vasopressors and type 2 diabetes mellitus, basically suffer from the same problem. Even in cell biology, variability and uncertainty in cellular responses to an identical excitation at the culture fluid level are inevitably regarded as random noise and abandoned from subsequent analysis, largely because they cannot be interpreted in the absence of a measure indicative of the actual excitation acting on the cells. It is obvious that, due to the absence of input observations, the standard inverse problem framework is not applicable to this class of systems and processes. Considering a wide range of real-world clinical applications associated with this class of biological processes (as listed above), developing novel methods and approaches to solve inverse problems for these systems can make enormous impacts on (i) advancing the scientific state-of-the-art as well as on (ii) expediting the developments in personalized medicine.

The goal of the proposed project is to develop and test the feasibility of a novel approach to solve the inverse problem for a class of systems arising from systems biology study, in which input is unknown (e.g. cannot be observed) but multiple outputs can be observed. As mentioned above, the most significant challenge associated with this class of inverse problems is that the standard approach to inverse problem (where the parameters of the mechanistic model are optimized to fit the input-output data) is not applicable in the absence of system input observation. To resolve this challenge, this project proposes to exploit the commonality shared by the outputs that originate from the same input. Indeed, noting that these outputs originate from the identical input, a relationship between these outputs can be formulated, which can subsequently be utilized in solving the inverse problem without necessitating the input observations.

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1. PROBLEM STATEMENT

In this project, a generic mechanistic model in which each input-output relationship is given by a cascade connection of a linear dynamic system, a static nonlinear function and an indirect response mechanism is considered. Consider Figure 1, in which the input to the system is unknown but multiple outputs from the system can be observed. In particular, this project considers systems with two output observations, which results in the most challenging inverse problem of this kind. The system is described by a class of mechanistic model called the indirect response model, used widely in systems biology at multiple different scales from cell signaling and receptor theory to quantitative pharmacology. In Figure 1, u is the input, R_j is the j -th response (output), $f_{ji}(x_j, \theta_{ji})$ and $f_{jo}(x_j, \theta_{jo})$ are the saturating nonlinear functions representing stimulation and/or inhibition effects of the input, k_{ji} and k_{jo} are the rate constants for production and loss of the response, and τ_j is the time constant associated with the distribution of the input to the site(s) of action:

$$\begin{aligned} \dot{R}_j &= k_{ji}f_{ji}(x_j, \theta_{ji}) - k_{jo}f_{jo}(x_j, \theta_{jo})R_j, \quad j = 1, 2 \\ \tau_j \dot{x}_j &= -x_j + u \end{aligned} \quad (1)$$

In this class of models, the input u is delayed by the latency model $\tau_j \dot{x}_j = -x_j + u$ to produce x_j . From the traditional pharmacological perspective, u and x_j can be regarded as the concentration of a medication agent in the blood and at the site of action, respectively. x_j is then translated to the stimulation and/or inhibition effects acting upon the production (through $f_{ji}(x_j, \theta_{ji})$) and dissipation (through $f_{jo}(x_j, \theta_{jo})$) of the response R_j . The resulting response R_j is then elicited by the model $\dot{R}_j = k_{ji}f_{ji}(x_j, \theta_{ji}) - k_{jo}f_{jo}(x_j, \theta_{jo})R_j$. This class of mechanistic models has been widely used in systems biology and pharmacology [10, 11], including mechanistic modeling studies in cancer chemotherapy (e.g., [12, 13]), anti-diabetic drugs (e.g., [14]) and HIV (e.g., [15]).

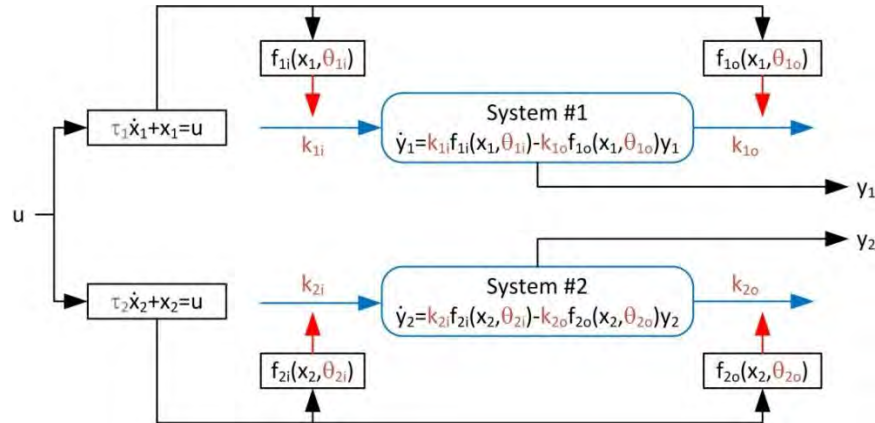


Figure 1: Systems biology process described by a class of mechanistic models. Two outputs are available as observations but input observation is not available. In this project, the inverse problem for this class of biological systems will be solved by exploiting the commonality shared by the output observations in the absence of input observations.

2. SUMMARY OF THE MOST IMPORTANT RESULTS

The goal of this project is to develop a novel approach to solve inverse problems for a class of systems biology models with unknown inputs, and test the validity and feasibility of the approach. The specific aims of the proposed project are to (i) develop a systematic inverse modeling procedure and (ii) to assess its preliminary feasibility with numerical example(s).

2.1. High-Level Overview

In this project, we achieve the above specific aims as follows. Based on the model shown in Figure 1, we consider 3 problems having increasing complexities: (i) steady-state dose-response problem (Figure 2a), (ii) indirect dose-response problem with immediate dose effect (Figure 2b), and (iii) indirect dose-response problem with latency in dose effect (Figure 2c). For each of these inverse problems, we first study the solvability of the problem in terms of the identifiability of model parameters. We elucidate the best extent to which the model parameters can be identified from two output observations, as well as the circumstance(s) under which this can be achieved. We then establish a step-by-step procedure to identify the model.

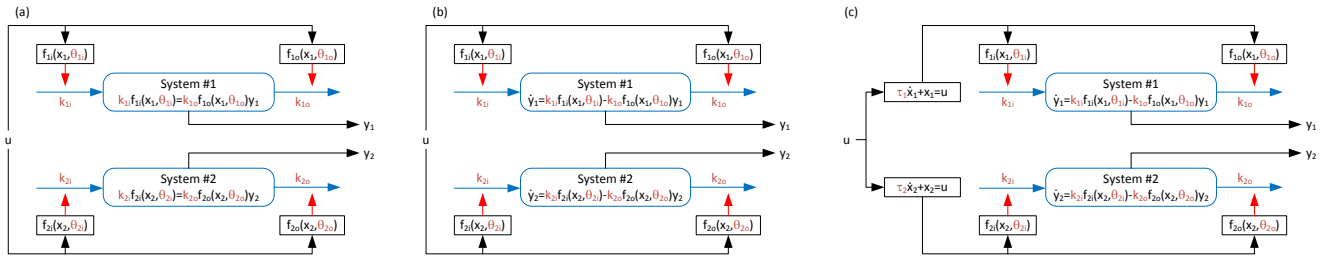


Figure 2: The class of inverse problem pursued in this project. (a) Steady-state dose-response problem. (b) Indirect dose-response problem with immediate dose effect. (c) Indirect dose-response problem with latency in dose effect.

2.2. Steady-State Dose-Response Problem

The steady-state dose-response problem is obtained by eliminating all the dynamic (i.e., time derivative) terms from Eq. (1):

$$k_{ji}f_{ji}(x_j, \theta_{ji}) = k_{jo}f_{jo}(x_j, \theta_{jo})R_j, \quad j = 1, 2 \quad (2)$$

$$x_j = u$$

In the systems biology models, the stimulation and inhibition effects are often given by the following saturation function:

$$f_{ji}(x_j, \theta_{ji}) = 1 \pm \frac{b_{ji}x_j}{a_{ji} + x_j}, \quad f_{jo}(x_j, \theta_{jo}) = 1 \pm \frac{b_{jo}x_j}{a_{jo} + x_j}, \quad j = 1, 2 \quad (3)$$

where $\theta_{ji} = \{a_{ji}, b_{ji}\}$ and $\theta_{jo} = \{a_{jo}, b_{jo}\}$. It is obvious that the “+” sign represents stimulation while the “-” sign represents inhibition. Besides, we assume that the initial values of the outputs $R_j(t = 0)$ before the application of the input (i.e., $u = 0$ for $t < 0$), are available. Since $f_{ji}(x_j, \theta_{ji}) = f_{jo}(x_j, \theta_{jo}) = 1$ if $u = 0$, we have:

$$k_{ji} = k_{jo}R_j, \quad j = 1, 2 \quad (4)$$

Combining Eq. (2)-(4) results in the following relation between x_j and R_j :

$$\left[1 \pm \frac{b_{ji}x_j}{a_{ji} + x_j}\right] R_j(0) = \left[1 \pm \frac{b_{jo}x_j}{a_{jo} + x_j}\right] R_j(t), \quad j = 1, 2 \quad (5)$$

Most often, the stimulation/inhibition effect acts only upon production (i.e., to k_{ji}) or dissipation (i.e., to k_{jo}). In case the effect acts upon production, Eq. (5) reduces to:

$$\left[1 \pm \frac{b_{ji}x_j}{a_{ji} + x_j}\right] R_j(0) = R_j(t), \quad j = 1,2 \quad (6a)$$

whereas in case the effect acts upon dissipation:

$$R_j(0) = \left[1 \pm \frac{b_{jo}x_j}{a_{jo} + x_j}\right] R_j(t), \quad j = 1,2 \quad (6b)$$

Therefore, any steady-state dose-response can be represented by the following unified relation:

$$y_j(t) = \left[1 \pm \frac{b_j x_j}{a_j + x_j}\right], \quad j = 1,2 \quad (7)$$

where $y_j(t) = R_j(t)/R_j(0)$ if stimulation/inhibition effect acts upon production, and $y_j(t) = R_j(0)/R_j(t)$ if the effect acts upon dissipation. Then, the inverse problem for steady-state dose-response problem reduces to solving the following relation for a_j and b_j , $j = 1,2$:

$$a_1 \frac{y_1 - 1}{\pm b_1 + 1 - y_1} = a_2 \frac{y_2 - 1}{\pm b_2 + 1 - y_2} \quad (8)$$

2.2.1. Identifiability Analysis

For the steady-state dose-response problem in Eq. (8), we make the following claim on the identifiability:

CLAIM 1: Identifiability of Steady-State Dose-Response Model

Consider the steady-state dose-response model in Eq. (8). Based on two output observations $y_j(t)$, $j = 1,2$, the following parameters can be identified:

$$\begin{aligned} \lambda_1 &= \frac{a_1 b_2}{a_2 b_1} \\ \lambda_2 &= \left(\frac{1}{a_1} - \frac{1}{a_2}\right) \frac{a_1}{b_1} \end{aligned} \quad (9)$$

PROOF 1: See Appendix 1.

Several physical insights can be obtained from CLAIM 1. First, the individual parameter values cannot be identified just by solving Eq. (8). This can also be understood by reformulating Eq. (8) as follows:

$$\frac{1}{y_1 - 1} = \lambda_1 \frac{1}{y_2 - 1} + \lambda_2 \quad (10)$$

So, the two parameters λ_1 and λ_2 are sufficient to specify the relation between $y_1 - 1$ and $y_2 - 1$. In many real-world problems, however, full effects are typically assumed for $f_{ji}(x_j, \theta_{ji})$ and $f_{jo}(x_j, \theta_{jo})$ (so that $b_1 = 1$ and/or $b_2 = 1$). In this regard, 3 practically useful scenarios can be conceived:

- 1) $b_j = 1$, $j = 1,2$: In this case, Eq. (9) returns a unique value of a_1/a_2 (see the discussion below).
- 2) $b_j = p$, $j = 1$ or $j = 2$: In this case, Eq. (9) returns a unique values of a_1/a_2 and $b_j \neq p$.
- 3) $b_1 = \sigma b_2$, σ known a priori: In this case, Eq. (9) returns a unique values of a_1/a_2 and b_j , $j = 1,2$.

Second, a_j , $j = 1,2$ cannot be uniquely determined just by solving Eq. (8). This indeed makes sense, since by the problem formulation the input to the system u is unknown. In fact, an important consequence of input being unknown is that its scale is also unknown. To illustrate, suppose the input was doubled in the steady-state dose-response model shown in Eq. (7) (i.e.,

$x_j \rightarrow 2x_j$). It is possible to keep $y_j(t)$ consistent simply by doubling a_j . This recognition indicates that the absolute values of a_j cannot be identified based on Eq. (8); rather, only the ratio between a_1 and a_2 , a_1/a_2 , can be identified.

2.2.2. Inverse Problem Solution Procedure

Given $R_j(t)$, $t = 1, \dots, N$ and $j = 1, 2$, the steady-state dose-response problem can be solved by the following procedure:

- 1) Form $y_j(t) = R_j(t)/R_j(0)$, $j = 1, 2$.
- 2) Form Eq. (10) and formulate into a least-squares problem to identify λ_1 and λ_2 :

$$\{\lambda_1^*, \lambda_2^*\} = \arg \min_{\{\lambda_1, \lambda_2\}} \left\| \frac{1}{y_1 - 1} - \lambda_1 \frac{1}{y_2 - 1} - \lambda_2 \right\|$$

2.3. Indirect Dose-Response Problem with Immediate Dose Effect

The indirect dose-response problem with immediate dose effect is obtained by eliminating the latency model $\tau_j \dot{x}_j = -x_j + u$ from Eq. (1):

$$\dot{R}_j = k_{ji}f_{ji}(x_j, \theta_{ji}) - k_{jo}f_{jo}(x_j, \theta_{jo})R_j, \quad j = 1, 2 \quad (11)$$

$x_j = u$

It is assumed that 1) the saturation function in Eq. (3) is used to represent the stimulation and inhibition effects, and that 2) the stimulation/inhibition effect acts only upon production or dissipation. In contrast to the steady-state dose-response problem in which the inverse problem reduces to the unified relation in Eq. (8) regardless of how the stimulation/inhibition effect acts upon the biological system (i.e., whether it acts upon production or dissipation), the indirect dose-response problem yields three relations depending on the nature of the stimulation/inhibition effect as follows.

- 1) If both indirect response outputs $R_1(t)$ and $R_2(t)$ are subject to stimulation/dissipation effect acting upon production, each indirect response model reduces to the following:

$$\dot{R}_j = k_{ji}f_{ji}(u, \theta_{ji}) - k_{jo}R_j, \quad j = 1, 2 \quad (12a)$$

- 2) If both indirect response outputs $R_1(t)$ and $R_2(t)$ are subject to stimulation/dissipation effect acting upon dissipation, each indirect response model reduces to the following:

$$\dot{R}_j = k_{ji} - k_{jo}f_{jo}(u, \theta_{jo})R_j, \quad j = 1, 2 \quad (12b)$$

- 3) If one indirect response output (say $R_1(t)$) is subject to stimulation/dissipation effect acting upon production while the other (say $R_2(t)$) is subject to stimulation/dissipation effect acting upon dissipation, the indirect response model reduces to the following:

$$\dot{R}_1 = k_{1i}f_{1i}(u, \theta_{1i}) - k_{1o}R_1, \quad \dot{R}_2 = k_{2i} - k_{2o}f_{2o}(u, \theta_{2o})R_2 \quad (12c)$$

Firstly, consider Eq. (12a). Using Eq. (3)-(4), Eq. (12a) reduces to

$$f_{ji}(u, \theta_{ji}) = 1 \pm \frac{b_j u}{a_j + u} = \frac{\dot{R}_j/k_{jo} + R_j}{R_{jo}} \triangleq \frac{\dot{y}_j}{k_{jo}} + y_j, \quad j = 1, 2 \quad (13)$$

where $y_j = R_j/R_{jo}$. So, solving Eq. (13) for u yields:

$$u = a_j \frac{\dot{z}_j/k_{jo} + z_j}{\pm b_j - \dot{z}_j/k_{jo} - z_j}, \quad j = 1, 2 \quad (14)$$

where $z_j = y_j - 1$. In sum, the inverse problem for indirect dose-response problem with immediate dose effect acting upon production reduces to solving the following relation for a_j , b_j and k_{jo} , $j = 1, 2$:

$$a_1 \frac{\dot{z}_1/k_{10} + z_1}{\pm b_1 - \dot{z}_1/k_{10} - z_1} = a_2 \frac{\dot{z}_2/k_{20} + z_2}{\pm b_2 - \dot{z}_2/k_{20} - z_2} \quad (15)$$

Secondly, consider Eq. (12b). Using Eq. (3)-(4), Eq. (12b) reduces to

$$f_{j0}(u, \theta_{j0}) = 1 \pm \frac{b_j u}{a_j + u} = \frac{k_{j0} - \dot{y}_j}{k_{j0} y_j}, \quad j = 1, 2 \quad (16)$$

where $y_j = R_j/R_{j0}$. So, solving Eq. (16) for u yields:

$$u = -a_j \frac{\dot{z}_j/k_{j0} + z_j}{\dot{z}_j/k_{j0} + z_j \pm b_j(1 + z_j)}, \quad j = 1, 2 \quad (17)$$

where $z_j = y_j - 1$. In sum, the inverse problem for indirect dose-response problem with immediate dose effect acting upon dissipation reduces to solving the following relation for a_j , b_j and k_{j0} , $j = 1, 2$:

$$a_1 \frac{\dot{z}_1/k_{10} + z_1}{\dot{z}_1/k_{10} + z_1 \pm b_1(1 + z_1)} = a_2 \frac{\dot{z}_2/k_{20} + z_2}{\dot{z}_2/k_{20} + z_2 \pm b_2(1 + z_2)} \quad (18)$$

Thirdly, consider Eq. (12c). Using Eq. (3)-(4), Eq. (14) and Eq. (17), Eq. (12c) reduces to

$$u = a_1 \frac{\dot{z}_1/k_{10} + z_1}{\pm b_1 - \dot{z}_1/k_{10} - z_1}, \quad u = -a_2 \frac{\dot{z}_2/k_{20} + z_2}{\dot{z}_2/k_{20} + z_2 \pm b_2(1 + z_2)} \quad (19)$$

In sum, the inverse problem for indirect dose-response problem with immediate dose effect acting upon production ($R_1(t)$) and dissipation ($R_2(t)$) reduces to solving the following relation for a_j , b_j and k_{j0} , $j = 1, 2$:

$$a_1 \frac{\dot{z}_1/k_{10} + z_1}{\pm b_1 - \dot{z}_1/k_{10} - z_1} = -a_2 \frac{\dot{z}_2/k_{20} + z_2}{\dot{z}_2/k_{20} + z_2 \pm b_2(1 + z_2)} \quad (20)$$

2.3.1. Identifiability Analysis

For each of the three relations presented in Eq. (12), we make the following claims on the identifiability:

CLAIM 2: Identifiability of Indirect Dose-Response Model with Immediate Dose Effect in Eq. (15)

Consider the indirect dose-response model with immediate dose effect in Eq. (12a). Based on two output observations $z_j(t)$, $j = 1, 2$, the following parameters can be identified:

$$\lambda_1 = \frac{a_1 b_2}{a_2 b_1}, \quad \lambda_2 = \left(\frac{1}{a_1} - \frac{1}{a_2} \right) \frac{a_1}{b_1}, \quad k_{10}, \quad k_{20} \quad (21)$$

PROOF 2: See Appendix 2.

CLAIM 3: Identifiability of Indirect Dose-Response Model with Immediate Dose Effect in Eq. (18)

Consider the indirect dose-response model with immediate dose effect in Eq. (12b). Based on two output observations $z_j(t)$, $j = 1, 2$, the following parameters can be identified:

$$\lambda_1 = \frac{a_1 b_2}{a_2 b_1}, \quad \lambda_2 = \left(\frac{1}{a_1} - \frac{1}{a_2} \right) \frac{a_1}{b_1}, \quad k_{10}, \quad k_{20} \quad (22)$$

PROOF 3: See Appendix 3.

CLAIM 4: Identifiability of Indirect Dose-Response Model with Immediate Dose Effect in Eq. (20)

Consider the indirect dose-response model with immediate dose effect in Eq. (12c). Based on two output observations $y_j(t)$, $j = 1, 2$, the following parameters can be identified:

$$\lambda_1 = \frac{a_1 b_2}{a_2 b_1}, \quad \lambda_2 = \left(\frac{1}{a_1} - \frac{1}{a_2} \right) \frac{a_1}{b_1}, \quad k_{10}, \quad k_{20} \quad (23)$$

PROOF 4: See Appendix 4.

It is noted that the models in Eq. (15), Eq. (18) and Eq. (20) above can be cast into a unified model of the following form:

$$\frac{1}{g_1(t)} = \lambda_1 \frac{1}{g_2(t)} + \lambda_2 \quad (24)$$

where $g_j(t)$, $j = 1, 2$ is given by

$$\frac{\dot{z}_j}{k_{j0}} + z_j \quad (25a)$$

in case of Eq. (15), whereas $g_j(t)$, $j = 1, 2$ is given by

$$-\left(\frac{1}{k_{j0}} \frac{\dot{z}_j}{z_j + 1} + \frac{z_j}{z_j + 1} \right) \quad (25b)$$

in case of Eq. (18). Accordingly, in case of Eq. (20), $g_j(t)$, $j = 1, 2$ is given by

$$g_1(t) = \frac{\dot{z}_1}{k_{10}} + z_1, \quad g_2(t) = -\left(\frac{1}{k_{20}} \frac{\dot{z}_2}{z_2 + 1} + \frac{z_2}{z_2 + 1} \right) \quad (25c)$$

It can be shown that, using Eq. (24), equivalent linear regression models for Eq. (15), Eq. (18) and Eq. (20) that contain λ_1 , λ_2 , k_{10} and k_{20} as unknown parameters can be obtained. For example, for Eq. (15), the following linear regression model can be derived from Eq. (24) and Eq. (25a):

$$z_2 = -\frac{1}{k_{20}} \dot{z}_2 + \frac{\lambda_1}{k_{10}} \dot{z}_1 + \lambda_1 z_1 + \frac{\lambda_2}{k_{10} k_{20}} \dot{z}_1 \dot{z}_2 + \frac{\lambda_2}{k_{10}} \dot{z}_1 z_2 + \frac{\lambda_2}{k_{20}} z_1 \dot{z}_2 + \lambda_2 z_1 z_2 \quad (26)$$

which clearly illustrates that the unknowns listed in Eq. (21) can be readily identified.

2.3.2. Inverse Problem Solution Procedure

Given $R_j(t)$, $t = 1, \dots, N$ and $j = 1, 2$, the indirect dose-response problem with immediate dose effect can be solved by the following procedure:

- 1) Form $y_j(t) = R_j(t)/R_j(0)$, $j = 1, 2$.
- 2) Using the steady-state dose-response data, form Eq. (10) and formulate into a least-squares problem to identify λ_1 and λ_2 :

$$\{\lambda_1^*, \lambda_2^*\} = \arg \min_{\{\lambda_1, \lambda_2\}} \left\| \frac{1}{y_1 - 1} - \lambda_1 \frac{1}{y_2 - 1} - \lambda_2 \right\|$$

- 3) Using the transient (or alternatively, all the) dose-response data, formulate Eq. (24) into a least-squares problem to identify k_{10} and k_{20} :

$$\{k_{10}^*, k_{20}^*\} = \arg \min_{\{k_{10}, k_{20}\}} \left\| \frac{1}{g_1(t; k_{10})} - \lambda_1 \frac{1}{g_2(t; k_{20})} - \lambda_2 \right\|$$

where $g_j(t)$, $j = 1, 2$ is given by Eq. (25).

2.4. Indirect Dose-Response Problem with Latency in Dose Effect

The indirect dose-response problem with latency in dose effect concerns Eq. (1). It is assumed that 1) the saturation function in Eq. (3) is used to represent the stimulation and inhibition effects, and that 2) the stimulation/inhibition effect acts only upon production or dissipation. Similarly to the indirect dose-response response problem with immediate dose effect, this problem yields three relations depending on the nature of the stimulation/inhibition effect as follows.

- 1) If both indirect response outputs $R_1(t)$ and $R_2(t)$ are subject to stimulation/dissipation effect acting upon production, each indirect response model reduces to the following:

$$\begin{aligned} \dot{R}_j &= k_{ji}f_{ji}(x_j, \theta_{ji}) - k_{jo}R_j, \quad j = 1,2 \\ \tau_j \dot{x}_j &= -x_j + u \end{aligned} \quad (27a)$$

- 2) If both indirect response outputs $R_1(t)$ and $R_2(t)$ are subject to stimulation/dissipation effect acting upon dissipation, each indirect response model reduces to the following:

$$\begin{aligned} \dot{R}_j &= k_{ji} - k_{jo}f_{jo}(x_j, \theta_{jo})R_j, \quad j = 1,2 \\ \tau_j \dot{x}_j &= -x_j + u \end{aligned} \quad (27b)$$

- 3) If one indirect response output (say $R_1(t)$) is subject to stimulation/dissipation effect acting upon production while the other (say $R_2(t)$) is subject to stimulation/dissipation effect acting upon dissipation, the indirect response model reduces to the following:

$$\begin{aligned} \dot{R}_1 &= k_{1i}f_{1i}(x_1, \theta_{1i}) - k_{1o}R_1, \quad \dot{R}_2 = k_{2i} - k_{2o}f_{2o}(x_2, \theta_{2o})R_2, \quad j = 1,2 \\ \tau_j \dot{x}_j &= -x_j + u \end{aligned} \quad (27c)$$

Firstly, consider Eq. (27a). Defining $z_j = y_j - 1$, $y_j = R_j/R_{jo}$ and $f_{ji}(x_j, \theta_{ji}) = 1 \pm b_j x_j / [a_j + x_j] = 1 + g_{ji}(x_j, \theta_{ji})$, the indirect dose-response model $\dot{R}_j = k_{ji}f_{ji}(x_j, \theta_{ji}) - k_{jo}R_j$ reduces to the following:

$$\dot{z}_j = k_{jo} [g_{ji}(x_j, \theta_{ji}) - z_j], \quad j = 1,2 \quad (28)$$

Then, x_j can be expressed in terms of z_j as follows:

$$x_j = \frac{a_j g_{ji}(x_j, \theta_{ji})}{\pm b_j - g_{ji}(x_j, \theta_{ji})} = \frac{a_j \left[\frac{\dot{z}_j}{k_{jo}} + z_j \right]}{\pm b_j - \left[\frac{\dot{z}_j}{k_{jo}} + z_j \right]}, \quad j = 1,2 \quad (29)$$

Therefore, it is possible to denote $g_{ji}(x_j, \theta_{ji}) = g_{ji}(z_j, k_{jo})$. Then, since $\dot{x}_j = a_j b_j \dot{g}_{ji}(z_j, k_{jo}) / [\pm b_j - g_{ji}(z_j, k_{jo})]^2$, the latency model can be rewritten as follows:

$$\frac{\tau_j a_j b_j \dot{g}_{ji}(z_j, k_{jo})}{[\pm b_j - g_{ji}(z_j, k_{jo})]^2} + \frac{a_j g_{ji}(z_j, k_{jo})}{\pm b_j - g_{ji}(z_j, k_{jo})} = u, \quad j = 1,2 \quad (30)$$

In sum, the inverse problem for indirect dose-response problem with latency in dose effect acting upon production reduces to solving the following relation for a_j , b_j , k_{jo} and τ_j , $j = 1,2$:

$$\frac{\tau_1 a_1 b_1 \dot{g}_{1i}(z_1, k_{1o})}{[\pm b_1 - g_{1i}(z_1, k_{1o})]^2} + \frac{a_1 g_{1i}(z_1, k_{1o})}{\pm b_1 - g_{1i}(z_1, k_{1o})} = \frac{\tau_2 a_2 b_2 \dot{g}_{2i}(z_2, k_{2o})}{[\pm b_2 - g_{2i}(z_2, k_{2o})]^2} + \frac{a_2 g_{2i}(z_2, k_{2o})}{\pm b_2 - g_{2i}(z_2, k_{2o})} \quad (31)$$

where

$$g_{ji}(z_j, k_{jo}) = \frac{\dot{z}_j}{k_{jo}} + z_j, \quad \dot{g}_{ji}(z_j, k_{jo}) = \frac{\ddot{z}_j}{k_{jo}} + \dot{z}_j \quad (32)$$

Secondly, consider Eq. (27b). Defining z_j and y_j as above and $f_{j0}(x_j, \theta_{j0}) = 1 \pm b_j x_j / [a_j + x_j] = 1 + g_{j0}(x_j, \theta_{j0})$, the indirect dose-response model $\dot{R}_j = k_{ji} - k_{j0} f_{j0}(x_j, \theta_{j0}) R_j$ reduces to the following:

$$\dot{z}_j = k_{j0} [g_{j0}(x_j, \theta_{j0}) y_j - z_j], \quad j = 1, 2 \quad (33)$$

Then, x_j can be expressed in terms of z_j as follows:

$$x_j = \frac{a_j g_{j0}(x_j, \theta_{j0})}{\mp b_j - g_{j0}(x_j, \theta_{j0})} = \frac{a_j \left[\frac{\dot{z}_j}{k_{j0}} + z_j \right]}{\mp b_j y_j - \left[\frac{\dot{z}_j}{k_{j0}} + z_j \right]} = \frac{a_j \left[\frac{\dot{z}_j}{k_{j0}} + z_j \right]}{\mp b_j (z_j + 1) - \left[\frac{\dot{z}_j}{k_{j0}} + z_j \right]}, \quad j = 1, 2 \quad (34)$$

Therefore, it is possible to denote $g_{j0}(x_j, \theta_{j0}) = g_{j0}(z_j, k_{j0})$. Then, since $\dot{x}_j = a_j b_j \dot{g}_{j0}(z_j, k_{j0}) / [\pm b_j - g_{j0}(z_j, k_{j0})]^2$, the latency model can be rewritten as follows:

$$\frac{\tau_j a_j b_j \dot{g}_{j0}(z_j, k_{j0})}{[\mp b_j - g_{j0}(z_j, k_{j0})]^2} + \frac{a_j g_{j0}(z_j, k_{j0})}{\mp b_j - g_{j0}(z_j, k_{j0})} = u, \quad j = 1, 2 \quad (35)$$

In sum, the inverse problem for indirect dose-response problem with latency in dose effect acting upon dissipation reduces to solving the following relation for a_j, b_j, k_{j0} and $\tau_j, j = 1, 2$:

$$\frac{\tau_1 a_1 b_1 \dot{g}_{10}(z_1, k_{10})}{[\mp b_1 - g_{10}(z_1, k_{10})]^2} + \frac{a_1 g_{10}(z_1, k_{10})}{\mp b_1 - g_{10}(z_1, k_{10})} = \frac{\tau_2 a_2 b_2 \dot{g}_{20}(z_2, k_{20})}{[\mp b_2 - g_{20}(z_2, k_{20})]^2} + \frac{a_2 g_{20}(z_2, k_{20})}{\mp b_2 - g_{20}(z_2, k_{20})} \quad (36)$$

where

$$g_{j0}(z_j, k_{j0}) = -\frac{\dot{z}_j + k_{j0} z_j}{k_{j0}(z_j + 1)}, \quad \dot{g}_{j0}(z_j, k_{j0}) = -\frac{\ddot{z}_j(z_j + 1) + k_{j0} \dot{z}_j - \dot{z}_j^2}{k_{j0}(z_j + 1)^2} \quad (37)$$

Thirdly, consider Eq. (27c). Using Eq. (30) and Eq. (35), Eq. (27c) reduces to

$$u = \frac{\tau_1 a_1 b_1 \dot{g}_{1i}(z_1, k_{10})}{[\pm b_1 - g_{1i}(z_1, k_{10})]^2} + \frac{a_1 g_{1i}(z_1, k_{10})}{\pm b_1 - g_{1i}(z_1, k_{10})}, \quad u = \frac{\tau_2 a_2 b_2 \dot{g}_{20}(z_2, k_{20})}{[\mp b_2 - g_{20}(z_2, k_{20})]^2} + \frac{a_2 g_{20}(z_2, k_{20})}{\mp b_2 - g_{20}(z_2, k_{20})} \quad (38)$$

In sum, the inverse problem for indirect dose-response problem with immediate dose effect acting upon production ($R_1(t)$) and dissipation ($R_2(t)$) reduces to solving the following relation for a_j, b_j and $k_{j0}, j = 1, 2$:

$$\frac{\tau_1 a_1 b_1 \dot{g}_{1i}(z_1, k_{10})}{[\pm b_1 - g_{1i}(z_1, k_{10})]^2} + \frac{a_1 g_{1i}(z_1, k_{10})}{\pm b_1 - g_{1i}(z_1, k_{10})} = \frac{\tau_2 a_2 b_2 \dot{g}_{20}(z_2, k_{20})}{[\mp b_2 - g_{20}(z_2, k_{20})]^2} + \frac{a_2 g_{20}(z_2, k_{20})}{\mp b_2 - g_{20}(z_2, k_{20})} \quad (39)$$

where

$$g_{1i}(z_1, k_{10}) = \frac{\dot{z}_1}{k_{10}} + z_1, \quad g_{20}(z_2, k_{20}) = -\frac{\dot{z}_2 + k_{20} z_2}{k_{20}(z_2 + 1)} \quad (40)$$

2.4.1. Identifiability Analysis

The models in Eq. (31), Eq. (36) and Eq. (39) can be cast into a unified model of the following form, which can be viewed as an expanded version of Eq. (24) that includes the latency in dose effect:

$$g_2(t) - \lambda_1 g_1(t) - \lambda_2 g_1(t) g_2(t) = \tau_1 \frac{a_1}{a_2} \dot{g}_1(t) \frac{b_2 - g_2(t)}{b_1 - g_1(t)} - \tau_2 \frac{b_2}{b_1} \dot{g}_2(t) \frac{b_1 - g_1(t)}{b_2 - g_2(t)} \quad (41)$$

where $g_j(t)$, $j = 1, 2$ is given by Eq. (25a) for Eq. (31), Eq. (25b) for Eq. (36), and Eq. (25c) for Eq. (39). Noting that $g_j(t)$ can be written as the following unified form:

$$g_j(t) = \frac{w_j}{k_{jo}} + v_j \quad (42)$$

where w_j and v_j can be constructed from measurements (and thus can be regarded as known), the identifiability of the indirect dose-response problem with latency in dose effect can be analyzed by investigating the identifiability property of Eq. (41). We make the following claim on the identifiability.

CLAIM 5: Identifiability of Indirect Dose-Response Model with Latency in Dose Effect

Consider the indirect dose-response model with latency in dose effect in Eq. (41). Based on two output observations $y_j(t)$, $j = 1, 2$, the following parameters can be identified:

$$\tau_1, \quad \tau_2, \quad k_{10}, \quad k_{20}, \quad b_1, \quad b_2, \quad \frac{a_1}{a_2} \quad (43)$$

PROOF 5: See Appendix 5.

2.4.2. Inverse Problem Solution Procedure

Given $R_j(t)$, $t = 1, \dots, N$ and $j = 1, 2$, the indirect dose-response problem with immediate dose effect can be solved by the following procedure:

- 1) Form $y_j(t) = R_j(t)/R_j(0)$, $j = 1, 2$.
- 2) Using the steady-state dose-response data, form Eq. (10) and formulate into a least-squares problem to identify λ_1 and λ_2 :

$$\{\lambda_1^*, \lambda_2^*\} = \arg \min_{\{\lambda_1, \lambda_2\}} \left\| \frac{1}{y_1 - 1} - \lambda_1 \frac{1}{y_2 - 1} - \lambda_2 \right\|$$

- 3) Using the transient (or alternatively, all the) dose-response data, formulate Eq. (41) into a least-squares problem to identify $\tau_1, \tau_2, k_{10}, k_{20}, b_1, b_2$ and a_1/a_2 :

$$\min \left\| g_2(t) - \lambda_1 g_1(t) - \lambda_2 g_1(t)g_2(t) - \tau_1 \frac{a_1}{a_2} \dot{g}_1(t) \frac{b_2 - g_2(t)}{b_1 - g_1(t)} + \tau_2 \frac{b_2}{b_1} \dot{g}_2(t) \frac{b_1 - g_1(t)}{b_2 - g_2(t)} \right\|$$

where $g_j(t)$, $j = 1, 2$ is given by Eq. (42).

3. BENCHMARK EXAMPLES

The efficacy of the proposed inverse modeling approach is demonstrated using 3 benchmark examples: bronchodilator, anti-cancer drug and vasopressor.

3.1. Terbutaline Dose-Response Modeling [16]

Terbutaline is a fast-acting bronchodilator, which is often used as short-term treatment aid for asthma. In a previous study, a pharmacological model dictating the effect of plasma concentration of terbutaline on the changes in glucose, insulin and potassium concentrations in healthy adults was developed. In this project, this model was adopted as a benchmark problem where the relations between the plasma concentration of terbutaline versus glucose and potassium concentrations were derived from the measurements of glucose and potassium. The model relating the plasma concentration of terbutaline (x) to glucose (R_1) is given by:

$$\dot{R}_1 = k_{1i} \left(1 + \frac{b_1 x}{a_1 + x} \right) - k_{1o} (1 + F(I)) R_1 \quad (44)$$

where $F(I)$ is a known function of insulin concentration I , while the model relating the plasma concentration of terbutaline to potassium is given by:

$$\dot{R}_2 = k_{2i} - k_{2o} \left(1 + \frac{b_2 x}{a_2 + x} \right) R_2 \quad (45)$$

So, the problem reduces to an indirect dose-response problem with immediate dose effect. The plasma concentration of terbutaline x was generated by using a pharmacokinetic model, which was derived by fitting a two-compartment model to the data reported in a previous study [16]. A set of escalating infusion rates was used to produce response data employed in solving the inverse problem (see Figure 3 for the time courses of terbutaline infusion rate, terbutaline plasma concentration, glucose concentration and potassium concentration). The “true” parameter values used in the simulation are summarized in Table 1.

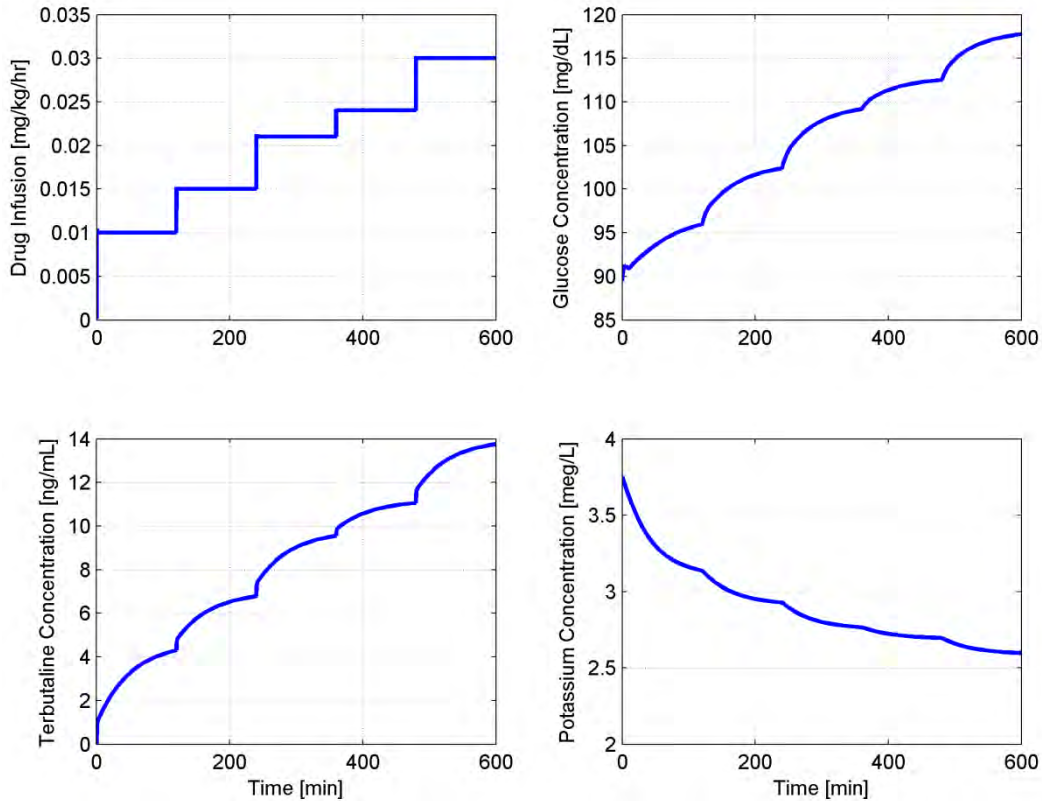


Figure 3: Time courses of terbutaline infusion rate, glucose concentration and potassium concentration.

Table 1: Parameter values used in terbutaline dose-response modeling and the corresponding estimates.

	λ_1	λ_2	k_{1o}	k_{2o}
True Values	1.3263	0.0995	0.0724	0.0324
Estimates	1.3263+/-0.0000	0.0995+/-0.0000	0.0724+/-0.0000	0.0324+/-0.0000

The inverse problem was solved using a MATLAB's numerical optimization function to yield the estimates of the parameters in Table 1. In solving the problem, a set of 20 random initial guesses for the parameters (within +/-30 % of each of the true parameter values in Table 1) was used to assess the robustness of the proposed inverse modeling approach. The resulting distributions of the estimates are summarized in Table 1.

3.2. Anti-Cancer Drug Dose-Response Modeling

cMet and hepatocyte growth factor (HGF) have been implicated in the development and progression of multiple human cancers and are attractive targets for cancer therapy. PF02341066 is a compound that was shown to be effective in inhibiting cMet phosphorylation and signal transduction as well as cMet-dependent proliferation, migration or invasion of human tumor cells, and also exhibits anti-angiogenic properties [17]. In a previous study, a pharmacological model that relates the plasma concentration of PF02341066 to cMet phosphorylation in tumor and tumor volume was developed [12]. In this project, this model was used as a benchmark problem where the pharmacological relations between the plasma concentration of PF02341066 versus cMet phosphorylation and tumor volume were derived from their measurements. The model relating the effect site concentration of PF02341066 (y_1) to cMet phosphorylation is given by:

$$\dot{R}_1 = k_{1i} \left(1 - \frac{b_1 y_1}{a_1 + y_1} \right) - k_{1o} R_1 \quad (46)$$

while the model relating the plasma concentration of PF02341066 (x) to tumor volume is given by:

$$\dot{R}_2 = k_{2i} \left(1 - \frac{b_2 x}{a_2 + x} \right) R_2 - k_{2o} R_2 = (k_{2i} - k_{2o}) \left[1 - \frac{(b_2 / (k_{2i} - k_{2o})) x}{a_2 + x} \right] R_2 \quad (47)$$

So, the problem reduces to an indirect dose-response problem with latency in dose effect in R_1 and immediate dose effect in R_2 . The plasma concentration of PF02341066 x was generated by using a pharmacokinetic model, which was derived by fitting a single compartment model with first-order absorption to the data reported in a previous study [12]. A set of impulse doses was used to produce response data employed in solving the inverse problem (see Figure 4 for the time courses of PF02341066 dose, PF02341066 plasma concentration, cMet phosphorylation and tumor volume). The "true" parameter values used in the simulation are summarized in Table 2.

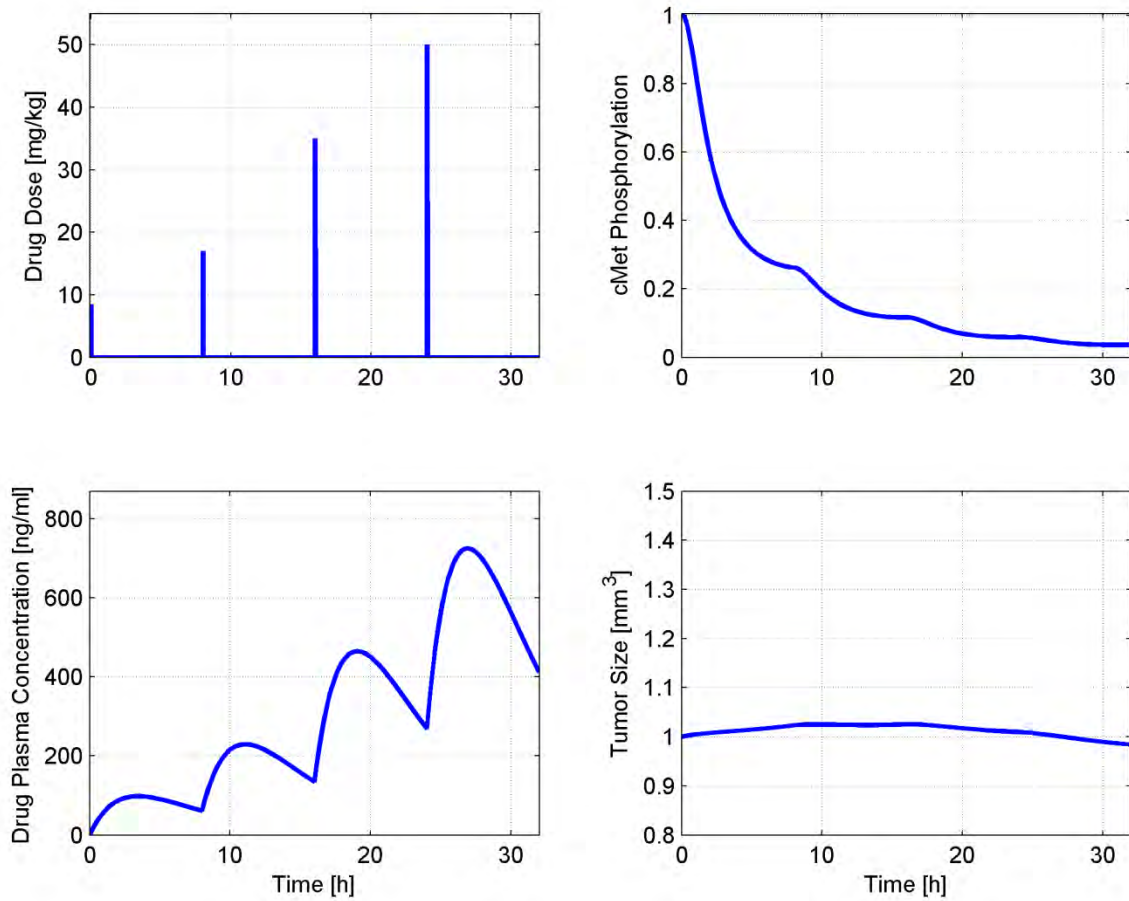


Figure 4: Time courses of PF02341066 dose, cMet phosphorylation and tumor volume.

Table 2: Parameter values used in PF02341066 dose-response modeling and the corresponding estimates.

	b_1	b_2	k_{10}	k_{20}	τ_1	a_2/a_1
True Values	1	2.070	20	0.0063	7.353	11.514
Estimates	1.0000 \pm 0.0000	2.0710 \pm 0.0001	20.019 \pm 0.0013	0.0063 \pm 0.0000	7.3672 \pm 0.0002	11.5176 \pm 0.0004

The inverse problem was solved using a MATLAB's numerical optimization function to yield the estimates of the parameters in Table 2. In solving the problem, a set of 20 random initial guesses for the parameters (within $\pm 30\%$ of each of the true parameter values in Table 2) was used to assess the robustness of the proposed inverse modeling approach. The resulting distributions of the estimates are summarized in Table 2.

3.3. Frusemide Dose-Response Modeling

The pharmacodynamics of frusemide in terms of diuresis and natriuresis can be modeled by indirect response model [18]. In this project, a modified version of this model was used as a benchmark problem where the pharmacological relations between the plasma concentration of frusemide versus its diuretic and natriuretic effects were derived from their measurements. The model relating the effect site excretion rate of frusemide (y_1) to diuresis is given by:

$$\dot{R}_1 = k_{1i} - k_{1o} \left(1 - \frac{b_1 y_1}{a_1 + y_1} \right) R_1 \quad (48)$$

while the model relating the effect site excretion rate of frusemide (y_2) to natriuresis is given by:

$$\dot{R}_2 = k_{2i} - k_{2o} \left(1 - \frac{b_2 y_2}{a_2 + y_2} \right) R_2 \quad (49)$$

So, the problem reduces to an indirect dose-response problem with latency in dose effects. The urinary excretion rate of frusemide x was generated by using a pharmacokinetic model, which was derived by fitting a three-compartment model to the data reported in a previous study [18]. A set of escalating infusion rates superimposed by a sequence of uniform random numbers was used to produce response data employed in solving the inverse problem (see Figure 5 for the time courses of frusemide infusion rate, frusemide urinary excretion rate, diuresis and natriuresis). The “true” parameter values used in the simulation are summarized in Table 3.

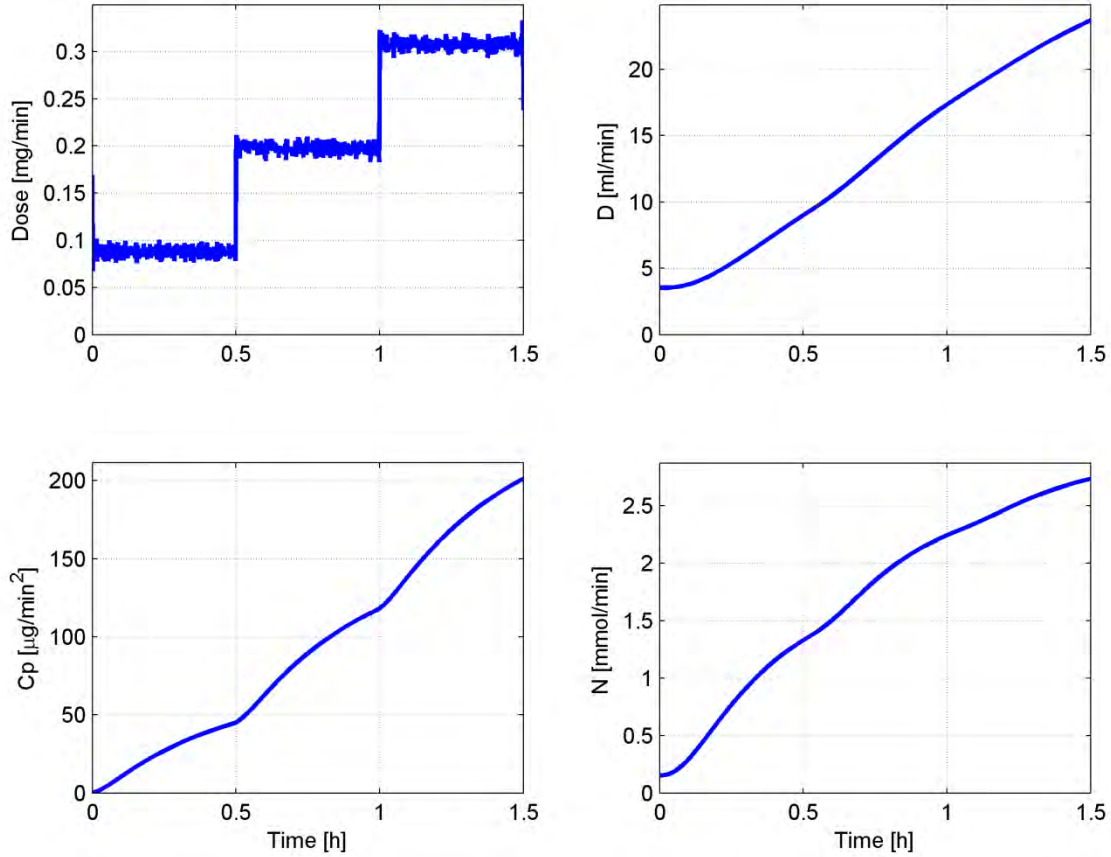


Figure 5: Time courses of frusemide infusion rate, diuresis and natriuresis.

Table 3: Parameter values used in frusemide dose-response modeling and the corresponding estimates: mean (SD).

	b_1	b_2	k_{1o}	k_{2o}	τ_1	τ_2	a_2/a_1
True Values	0.9100	0.9600	74	260	0.4762	0.0989	0.3649
Estimates	0.9089+/- 0.0021	0.9596+/- 0.0007	72.7886+/- 1.3256	261.2992+/- 6.6937	0.4881+/- 0.0106	0.1026+/- 0.0025	0.3621+/- 0.0006

The inverse problem was solved using a MATLAB's numerical optimization function to yield the estimates of the parameters in Table 3. In solving the problem, a set of 20 random initial guesses for the parameters (within +/-30 % of each of the true

parameter values in Table 3) was used to assess the robustness of the proposed inverse modeling approach. The resulting distributions of the estimates are summarized in Table 3.

4. Conclusions

This study proposed and demonstrated the feasibility of deriving data-driven model of a class of mechanistic biological system models from output responses alone. Mathematical analysis was performed to elucidate the identifiability property of the approach, which was then validated with benchmark numerical examples. In sum, the approach proposed in this study (called the “endpoint information fusion”) can be very useful in deriving data-driven models of a class of mechanistic biological system models, which is potentially applicable to wide-ranging systems biology investigations.

This study leaves room for open challenges that need to be rigorously addressed in the follow-up studies. First, this study showed that filtered white noise is effective in deriving high-fidelity data-based models (especially the parameters in the model responsible for dynamic responses, i.e., effect compartment time constant). However, no systematic analysis was performed in this study to understand how to select/optimize dynamic inputs required to derive high-fidelity data-based models. Future studies to address this issue need to be conducted. Second, this study showed the feasibility of batch inverse modeling based on the endpoint information fusion approach. In reality, however, the underlying biology varies as the system profile varies. Future studies on recursive inverse modeling based on the endpoint information fusion approach are thus warranted. Third, this study examined the endpoint information fusion approach via simulation to establish its initial proof-of-principle. To make a strong claim on its efficacy, follow-up work on experimental validation of the approach must be conducted.

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APPENDIXES

Appendix 1. Proof of Claim 1: Identifiability of Steady-State Dose-Response Model

Starting from Eq. (8) we write:

$$a_1 \frac{y_1 - 1}{\pm b_1 + 1 - y_1} = a_2 \frac{y_2 - 1}{\pm b_2 + 1 - y_2} \quad (\text{A.1})$$

Inverting both sides we have:

$$\frac{1}{a_1} \frac{\pm b_1 + 1 - y_1}{y_1 - 1} = \frac{1}{a_2} \frac{\pm b_2 + 1 - y_2}{y_2 - 1} \quad (\text{A.2})$$

Next multiplying both sides by a_1 we may write:

$$\frac{\pm b_1}{y_1 - 1} = \frac{a_1}{a_2} \frac{\pm b_2}{y_2 - 1} + \left(1 - \frac{a_1}{a_2}\right) \quad (\text{A.3})$$

And then we divide by $\pm b_1$:

$$\frac{1}{y_1 - 1} = \frac{a_1}{a_2} \frac{b_2}{b_1} \frac{1}{y_2 - 1} + \left(\frac{1}{a_1} - \frac{1}{a_2}\right) \frac{a_1}{b_1} \quad (\text{A.4})$$

Finally, by denoting $\frac{a_1}{a_2} \frac{b_2}{b_1} = \lambda_1$ and $\left(\frac{1}{a_1} - \frac{1}{a_2}\right) \frac{a_1}{b_1} = \lambda_2$ we arrive at the linear parametric model:

$$Y = \Phi^T \theta \quad (\text{A.5})$$

in which the regressor vector is defined as:

$$\Phi = \left[\frac{1}{y_2 - 1} \quad 1 \right]^T \quad (\text{A.6})$$

and the unknown parameter vector is defined as:

$$\theta = [\lambda_1 \quad \lambda_2]^T \quad (\text{A.7})$$

So, θ is identifiable as long as informative data are provided to solve Eq. (A.5).

Appendix 2. Proof of Claim 2: Identifiability of Indirect Dose-Response Model with Immediate Dose Effect in Eq. (13)

Starting from Eq. (13) we write:

$$\frac{a_1}{a_2} \frac{\dot{z}_1/k_{10} + z_1}{\pm b_1 - \dot{z}_1/k_{10} - z_1} = \frac{\dot{z}_2/k_{20} + z_2}{\pm b_2 - \dot{z}_2/k_{20} - z_2} \quad (\text{A.8})$$

By multiplying both sides of Eq. (A.8) by the product of the denominators, we have:

$$\frac{a_1}{a_2} (\pm b_2) \left(\frac{\dot{z}_1}{k_{10}} + z_1 \right) \mp b_1 \left(\frac{\dot{z}_2}{k_{20}} + z_2 \right) + \left(1 - \frac{a_1}{a_2}\right) \left(\frac{\dot{z}_1 \dot{z}_2}{k_{10} k_{20}} + \frac{\dot{z}_1 z_2}{k_{10}} + \frac{z_1 \dot{z}_2}{k_{20}} + z_1 z_2 \right) = 0 \quad (\text{A.9})$$

Next, by expanding Eq. (A.9) and collecting terms of z_1 and z_2 and their derivatives, we have:

$$\begin{aligned} & \frac{b_1}{k_{20}} (\mp \dot{z}_2) + b_1 (\mp z_2) + \frac{a_1}{a_2} b_2 \frac{1}{k_{10}} (\pm \dot{z}_1) + \frac{a_1}{a_2} b_2 (\pm z_1) + \left(\frac{1}{a_1} - \frac{1}{a_2}\right) \frac{a_1}{k_{10} k_{20}} \dot{z}_1 \dot{z}_2 \\ & + \left(\frac{1}{a_1} - \frac{1}{a_2}\right) \frac{a_1}{k_{10}} \dot{z}_1 z_2 + \left(\frac{1}{a_1} - \frac{1}{a_2}\right) \frac{a_1}{k_{20}} z_1 \dot{z}_2 + \left(\frac{1}{a_1} - \frac{1}{a_2}\right) a_1 z_1 z_2 = 0 \end{aligned} \quad (\text{A.10})$$

Next, we multiply Eq. (A.15) by k_{20}/b_1 to achieve the linear parametric formulation:

$$\begin{aligned}
& (\mp \dot{z}_2) + k_{20}(\mp z_2) + \frac{a_1 b_2}{a_2 b_1} \frac{k_{20}}{k_{10}} (\pm \dot{z}_1) + \frac{a_1 b_2}{a_2 b_1} k_{20} (\pm z_1) + \left(\frac{1}{a_1} - \frac{1}{a_2} \right) \frac{a_1}{k_{10} b_1} \dot{z}_1 \dot{z}_2 \\
& + \left(\frac{1}{a_1} - \frac{1}{a_2} \right) \frac{a_1}{k_{10} b_1} k_{20} \dot{z}_1 z_2 + \left(\frac{1}{a_1} - \frac{1}{a_2} \right) \frac{a_1}{b_1} z_1 \dot{z}_2 + \left(\frac{1}{a_1} - \frac{1}{a_2} \right) \frac{a_1}{b_1} k_{20} z_1 z_2 = 0
\end{aligned} \tag{A.11}$$

By using $\lambda_1 = a_1 b_2 / a_2 b_1$ and $\lambda_2 = \left(\frac{1}{a_1} - \frac{1}{a_2} \right) \frac{a_1}{b_1}$, we may write:

$$k_{20}(\mp z_2) + \lambda_1 \frac{k_{20}}{k_{10}} (\pm \dot{z}_1) + \lambda_1 k_{20} (\pm z_1) + \frac{\lambda_2}{k_{10}} \dot{z}_1 \dot{z}_2 + \lambda_2 \frac{k_{20}}{k_{10}} \dot{z}_1 z_2 + \lambda_2 z_1 \dot{z}_2 + \lambda_2 k_{20} z_1 z_2 = \pm \dot{z}_2 \tag{A.12}$$

So, we arrive at the linear parametric model:

$$Y = \pm \dot{z}_2 = \phi^T \theta \tag{A.13}$$

where the regressor vector is defined as:

$$\phi = [\mp z_2 \quad \pm \dot{z}_1 \quad \pm z_1 \quad \dot{z}_1 \dot{z}_2 \quad \dot{z}_1 z_2 \quad z_1 \dot{z}_2 \quad z_1 z_2] \tag{A.14}$$

and the unknown parameter vector is defined as:

$$\theta = \left[k_{20} \quad \lambda_1 \frac{k_{20}}{k_{10}} \quad \lambda_1 k_{20} \quad \frac{\lambda_2}{k_{10}} \quad \lambda_2 \frac{k_{20}}{k_{10}} \quad \lambda_2 \quad \lambda_2 k_{20} \right] \tag{A.15}$$

So, θ is identifiable as long as informative data are provided to solve Eq. (A.13). Thus, λ_1 , λ_2 , k_{10} and k_{20} are identifiable as long as informative data are provided to solve Eq. (A.13).

Appendix 3. Proof of Claim 3: Identifiability of Indirect Dose-Response Model with Immediate Dose Effect in Eq. (16)

Starting from Eq. (18) we write:

$$\frac{a_1}{a_2} \frac{\dot{z}_1 / k_{10} + z_1}{\dot{z}_1 / k_{10} + z_1 \pm b_1(1 + z_1)} = \frac{\dot{z}_2 / k_{20} + z_2}{\dot{z}_2 / k_{20} + z_2 \pm b_2(1 + z_2)} \tag{A.16}$$

By multiplying both sides by the product of the denominators of the two fractions, we have:

$$\begin{aligned}
& \left(\frac{a_1}{a_2} - 1 \right) \left(\frac{\dot{z}_1 \dot{z}_2}{k_{10} k_{20}} + \frac{\dot{z}_1 z_2}{k_{10}} + \frac{z_1 \dot{z}_2}{k_{20}} + z_1 z_2 \right) \pm b_2 \frac{a_1}{a_2} \left(\frac{\dot{z}_1}{k_{10}} + \frac{\dot{z}_1 z_2}{k_{10}} + z_1 + z_1 z_2 \right) = \\
& \pm b_1 \left(\frac{\dot{z}_2}{k_{20}} + \frac{\dot{z}_2 z_1}{k_{20}} + z_2 + z_1 z_2 \right)
\end{aligned} \tag{A.17}$$

Now upon expanding, dividing both sides by b_1 , and using $\lambda_1 = \frac{a_1 b_2}{a_2 b_1}$ and $\lambda_2 = \left(\frac{1}{a_2} - \frac{1}{a_1} \right) \frac{a_1}{b_1}$ we may write:

$$\begin{aligned}
& \frac{\lambda_2}{k_{10} k_{20}} \dot{z}_1 \dot{z}_2 + \frac{\lambda_2}{k_{10}} \dot{z}_1 z_2 + \frac{\lambda_2}{k_{20}} z_1 \dot{z}_2 + \lambda_2 z_1 z_2 \pm \frac{\lambda_1}{k_{10}} (\dot{z}_1 + \dot{z}_1 z_2) + \lambda_1 (z_1 + z_1 z_2) \\
& \mp \frac{1}{k_{20}} (\dot{z}_2 + \dot{z}_2 z_1) = z_2 + z_1 z_2
\end{aligned} \tag{A.18}$$

So, we arrive at the linear parametric model:

$$Y = z_2 + z_1 z_2 = \phi^T \theta \tag{A.19}$$

where the regressor vector is defined as:

$$\phi = [\dot{z}_1 \dot{z}_2 \quad \dot{z}_1 z_2 \quad z_1 \dot{z}_2 \quad z_1 z_2 \quad \pm (\dot{z}_1 + \dot{z}_1 z_2) \quad (z_1 + z_1 z_2) \quad \mp (\dot{z}_2 + \dot{z}_2 z_1)] \tag{A.20}$$

and the unknown parameter vector is defined as:

$$\theta = \left[\frac{\lambda_2}{k_{10}k_{20}} \quad \frac{\lambda_2}{k_{10}} \quad \frac{\lambda_2}{k_{20}} \quad \lambda_2 \quad \frac{\lambda_1}{k_{10}} \quad \lambda_1 \quad \frac{1}{k_{20}} \right] \quad (\text{A.21})$$

So, θ is identifiable as long as informative data are provided to solve Eq. (A.19). Thus, λ_1 , λ_2 , k_{10} and k_{20} are identifiable.

Appendix 4. Proof of Claim 4: Identifiability of Indirect Dose-Response Model with Immediate Dose Effect in Eq. (18)

Starting from Eq. (20) we write:

$$\frac{a_1}{a_2 \pm b_1 - \dot{z}_1/k_{10} - z_1} = - \frac{\dot{z}_2/k_{20} + z_2}{\dot{z}_2/k_{20} + z_2 \pm b_2(1 + z_2)} \quad (\text{A.22})$$

By multiplying both sides by the product of the denominators of the two fractions, we have:

$$\frac{a_1}{a_2} \left(\frac{\dot{z}_1}{k_{10}} + z_1 \right) \left(\frac{\dot{z}_2}{k_{20}} + z_2 \pm b_2(1 + z_2) \right) = \left(\frac{\dot{z}_1}{k_{10}} + z_1 \mp b_1 \right) \left(\frac{\dot{z}_2}{k_{20}} + z_2 \right) \quad (\text{A.23})$$

Next, by expanding both sides and using $\lambda_1 = \frac{a_1 b_2}{a_2 b_1}$ and $\lambda_2 = \left(\frac{1}{a_2} - \frac{1}{a_1} \right) \frac{a_1}{b_1}$, we have:

$$\begin{aligned} \frac{\lambda_2}{k_{10}k_{20}} \dot{z}_1 \dot{z}_2 + \frac{\lambda_2}{k_{10}} \dot{z}_1 z_2 + \frac{\lambda_2}{k_{20}} \dot{z}_2 z_1 + \lambda_2 z_1 z_2 + \frac{\lambda_1}{k_{10}} (\pm (\dot{z}_1 + \dot{z}_1 z_2)) + \lambda_1 (\pm (z_1 + z_1 z_2)) + \frac{1}{k_{20}} (\mp \dot{z}_2) \\ = \mp \dot{z}_2 \end{aligned} \quad (\text{A.24})$$

So, we arrive at the linear parametric model:

$$Y = \mp \dot{z}_2 = \phi^T \theta \quad (\text{A.25})$$

where the regressor vector is defined as:

$$\phi = [\dot{z}_1 \dot{z}_2 \quad \dot{z}_1 z_2 \quad \dot{z}_2 z_1 \quad z_1 z_2 \quad \pm (\dot{z}_1 + \dot{z}_1 z_2) \quad \pm (z_1 + z_1 z_2) \quad \mp \dot{z}_2] \quad (\text{A.26})$$

and the unknown parameter vector is defined as:

$$\theta = \left[\frac{\lambda_2}{k_{10}k_{20}} \quad \frac{\lambda_2}{k_{10}} \quad \frac{\lambda_2}{k_{20}} \quad \lambda_2 \quad \frac{\lambda_1}{k_{10}} \quad \lambda_1 \quad \frac{1}{k_{20}} \right]^T \quad (\text{A.27})$$

So, θ is identifiable as long as informative data are provided to solve Eq. (A.25). Thus, λ_1 , λ_2 , k_{10} and k_{20} are identifiable.

Appendix 5. Proof of Claim 5: Identifiability of Indirect Dose-Response Model with Latency in Dose Effect

Starting from (41):

$$g_2(t) - \lambda_1 g_1(t) - \lambda_2 g_1(t)g_2(t) = \tau_1 \frac{a_1}{a_2} \dot{g}_1(t) \frac{b_2 - g_2(t)}{b_1 - g_1(t)} - \tau_2 \frac{b_2}{b_1} \dot{g}_2(t) \frac{b_1 - g_1(t)}{b_2 - g_2(t)} \quad (\text{A.28})$$

Multiplying both sides by $(b_1 - g_1(t))(b_2 - g_2(t))$ yields:

$$(g_2 - \lambda_1 g_1 - \lambda_2 g_1 g_2)(b_1 - g_1)(b_2 - g_2) = \tau_1 \frac{a_1}{a_2} \dot{g}_1 (b_2 - g_2)^2 - \tau_2 \frac{b_2}{b_1} \dot{g}_2 (b_1 - g_1)^2 \quad (\text{A.29})$$

which is expanded to the following:

$$\begin{aligned} (g_2 - \lambda_1 g_1 - \lambda_2 g_1 g_2)(b_1 b_2 - b_1 g_2 - b_2 g_1 - g_2 g_1) \\ = \tau_1 \frac{a_1}{a_2} \dot{g}_1 (b_2^2 + g_2^2 - 2b_2 g_2) - \tau_2 \frac{b_2}{b_1} \dot{g}_2 (b_1^2 + g_1^2 - 2b_1 g_1) \end{aligned} \quad (\text{A.30})$$

which can be rearranged to the following:

$$\begin{aligned}
& (b_1 b_2) g_2 - b_1 g_2^2 - \lambda_1 b_1 b_2 g_1 + (\lambda_1 b_1 - b_2 - b_1 b_2 \lambda_2) g_1 g_2 + \lambda_1 b_2 g_1^2 + (\lambda_1 + b_2 \lambda_2) g_2 g_1^2 \\
& + (\lambda_2 b_1 - 1) g_1 g_2^2 + \lambda_2 g_1^2 g_2^2 \\
& = \tau_1 \frac{a_1}{a_2} \dot{g}_1 (b_2^2 + g_2^2 - 2b_2 g_2) - \tau_2 \frac{b_2}{b_1} \dot{g}_2 (b_1^2 + g_1^2 - 2b_1 g_1)
\end{aligned} \tag{A.31}$$

Now dividing both sides of (A.36) by $\tau_2 b_2 b_1$ yields:

$$\begin{aligned}
& \left(\frac{1}{\tau_2} \right) g_2 - \left(\frac{1}{b_2 \tau_2} \right) g_2^2 - \frac{\lambda_1}{\tau_2} g_1 + \left(\frac{\lambda_1}{\tau_2 b_2} - \frac{1}{\tau_2 b_1} - \frac{\lambda_2}{\tau_2} \right) g_1 g_2 + \frac{\lambda_1}{\tau_2 b_1} g_1^2 + \frac{\lambda_1 + b_2 \lambda_2}{\tau_2 b_1 b_2} g_2 g_1^2 \\
& + \frac{(\lambda_2 b_1 - 1)}{\tau_2 b_1 b_2} g_1 g_2^2 + \frac{\lambda_2}{\tau_2 b_1 b_2} g_1^2 g_2^2 \\
& = \frac{\tau_1 a_1 b_2}{\tau_2 a_2 b_1} \dot{g}_1 + \frac{\tau_1 a_1}{\tau_2 a_2 b_1 b_2} \dot{g}_1 g_2^2 - 2 \frac{\tau_1 a_1}{\tau_2 a_2 b_1} \dot{g}_1 g_2 - \dot{g}_2 - \frac{1}{b_1^2} \dot{g}_2 g_1^2 + \frac{2}{b_1} \dot{g}_2 g_1
\end{aligned} \tag{A.32}$$

Next expanding g_1 and g_2 and using (42), (A.32) reduces to:

$$\begin{aligned}
& \frac{w_2}{k_{20} \tau_2} + \frac{v_2}{\tau_2} - \left(\frac{1}{b_2 \tau_2} \right) \left(\frac{w_2^2}{k_{20}^2} + v_2^2 + \frac{2w_2 v_2}{k_{20}} \right) - \frac{\lambda_1}{\tau_2} \left(\frac{w_1}{k_{10}} + v_1 \right) \\
& + \left(\frac{\lambda_1}{\tau_2 b_2} - \frac{1}{\tau_2 b_1} - \frac{\lambda_2}{\tau_2} \right) \left(\frac{w_1 w_2}{k_{10} k_{20}} + \frac{w_1 v_2}{k_{10}} + \frac{w_2 v_1}{k_{20}} + v_1 v_2 \right) + \frac{\lambda_1}{\tau_2 b_1} \left(\frac{w_1^2}{k_{10}^2} + v_1^2 + \frac{2w_1 v_1}{k_{10}} \right) \\
& + \frac{\lambda_1 + b_2 \lambda_2}{\tau_2 b_1 b_2} \left(\frac{w_1^2 w_2}{k_{10}^2 k_{20}} + \frac{w_1^2 v_2}{k_{10}^2} + \frac{v_1^2 w_2}{k_{20}} + v_1^2 v_2 + \frac{2w_1 v_1 w_2}{k_{10} k_{20}} + \frac{2w_1 v_1 v_2}{k_{10}} \right) \\
& + \frac{(\lambda_2 b_1 - 1)}{\tau_2 b_1 b_2} \left(\frac{w_2^2 w_1}{k_{20}^2 k_{10}} + \frac{w_2^2 v_1}{k_{20}^2} + \frac{v_2^2 w_1}{k_{10}} + v_2^2 v_1 + \frac{2w_1 v_2 w_2}{k_{10} k_{20}} + \frac{2w_2 v_1 v_2}{k_{20}} \right) \\
& + \frac{\lambda_2}{\tau_2 b_1 b_2} \left(\frac{w_1^2 w_2^2}{k_{10}^2 k_{20}^2} + \frac{w_1^2 v_2^2}{k_{10}^2} + \frac{2w_1^2 w_2 v_2}{k_{10}^2 k_{20}} + \frac{w_2^2 v_1^2}{k_{20}^2} + v_1^2 v_2^2 + \frac{2w_2 v_1^2 v_2}{k_{20}} + \frac{2w_1 w_2^2 v_1}{k_{10} k_{20}^2} + \frac{2w_1 v_1 v_2^2}{k_{10}} \right. \\
& \quad \left. + \frac{4w_1 w_2 v_1 v_2}{k_{10} k_{20}} \right) \\
& - \frac{\tau_1 a_1 b_2}{\tau_2 a_2 b_1} \left(\frac{\dot{w}_1}{k_{10}} + \dot{v}_1 \right) - \frac{\tau_1 a_1}{\tau_2 a_2 b_1 b_2} \left(\frac{\dot{w}_1 w_2^2}{k_{10} k_{20}^2} + \frac{\dot{w}_1 v_2^2}{k_{10}} + \frac{2\dot{w}_1 w_2 v_2}{k_{10} k_{20}} + \frac{\dot{v}_1 w_2^2}{k_{20}^2} + \dot{v}_1 v_2^2 + \frac{2\dot{v}_1 w_2 v_2}{k_{20}} \right) \\
& + 2 \frac{\tau_1 a_1}{\tau_2 a_2 b_1} \left(\frac{\dot{w}_1 w_2}{k_{10} k_{20}} + \frac{\dot{w}_1 v_2}{k_{10}} + \frac{\dot{v}_1 w_2}{k_{20}} + \dot{v}_1 v_2 \right) + \frac{\dot{w}_2}{k_{20}} \\
& + \frac{1}{b_1^2} \left(\frac{\dot{w}_2 w_1^2}{k_{20} k_{10}^2} + \frac{\dot{w}_2 v_1^2}{k_{20}} + \frac{2\dot{w}_2 w_1 v_1}{k_{10} k_{20}} + \frac{\dot{v}_2 w_1^2}{k_{10}^2} + \dot{v}_2 v_1^2 + \frac{2\dot{v}_2 w_1 v_1}{k_{10}} \right) - \frac{2}{b_1} \left(\frac{\dot{w}_2 w_1}{k_{10} k_{20}} + \frac{\dot{w}_2 v_1}{k_{20}} + \frac{\dot{v}_2 w_1}{k_{10}} + \dot{v}_2 v_1 \right) \\
& = -\dot{v}_2
\end{aligned} \tag{A.33}$$

So, we arrive at the linear parametric model:

$$Y = -\dot{v}_2 = \Phi^T \theta \tag{A.34}$$

Further expansion of (A.34) yields:

$$-\dot{v}_2 = \Phi_1^T \theta_1 + \Phi_2^T \theta_2 \tag{A.35}$$

Where:

$$\phi_1^T \theta_1 = [w_2, v_2, v_2^2, v_1, w_1, \dot{v}_2 v_1, \dot{v}_1 v_2] \left[\frac{1}{k_{20} \tau_2}, \frac{1}{\tau_2}, -\frac{1}{b_2 \tau_2}, -\frac{\lambda_1}{\tau_2}, -\frac{\lambda_1}{\tau_2 k_{10}}, -\frac{2}{b_1}, 2 \frac{\tau_1}{\tau_2} \frac{a_1}{a_2 b_1} \right]^T \quad (\text{A.36})$$

Note that $\phi_2^T \theta_2$ contains all the other terms in (A.33). From (A.36) we can clearly see that the parameter set (A.37) is uniquely identifiable:

$$\Theta = \left\{ k_{10}, k_{20}, b_1, b_2, \tau_1, \tau_2, \frac{a_2}{a_1} \right\} \quad (\text{A.37})$$